APPENDIX 3.8.~~6~~7

~~GUIDELINES FOR THE SURVEILLANCE REQUIRED TO SUPPORT THE ESTABLISHMENT OR REGAINING OF RECOGNITION FOR A FOOT AND MOUTH DISEASE FREE COUNTRY OR ZONE~~

GUIDELINES FOR THE SURVEILLANCE OF FOOT AND MOUTH DISEASE

Article 3.8.~~6~~7.1.

Introduction

This document defines the principles and provides a guide for the surveillance of foot and mouth disease (FMD) in accordance with Appendix 3.8.1. applicable to countries seeking recognition from the OIE for freedom from FMD, either with or without the use of vaccination. This may be for the entire country or a *zone* or *compartment* within the country. Guidance for countries seeking reestablishment of freedom from FMD for the whole country or a *zone* or a *compartment*, either with or without vaccination, following an *outbreak*, as well as guidelines for the maintenance of FMD status are ~~is~~ provided. These guidelines are intended to expand on and explain the requirements of Chapter 2.2.10. Applications to the OIE for ~~such~~ recognition of freedom should follow the format and answer all the questions posed by the "Questionnaire on FMD" available from the OIE Central Bureau.

~~Reference to vaccination in this guide implies vaccination as part of an official disease control programme under the supervision of the *Veterinary Administration* aimed at interrupting the transmission of FMD virus (FMDV) in the zone or country concerned. The level of herd immunity required to achieve interruption of transmission will depend on the size, composition (e.g. species) and density of the susceptible population. It is therefore impossible to be prescriptive in this matter but, in general, unless there are good reasons to employ a different target, the aim should be to vaccinate at least 80% of the susceptible population in the manner and at the frequency prescribed by the manufacturer of the vaccine concerned. The vaccine must also comply with the provisions stipulated for FMD vaccines in the *Terrestrial Manual*. It may be that a decision is reached to vaccinate only certain species or other subset of the total susceptible population. In that case the rationale should be contained within the dossier accompanying the application to the OIE for recognition of a free country or zone or recovery of such status.~~

The impact and epidemiology of FMD differ widely in different regions of the world and therefore it is impossible to provide specific guidelines for all situations. It is axiomatic that the surveillance strategies employed for demonstrating freedom from FMD at an acceptable level of confidence will need to be adapted to the local situation. For example, the approach to proving freedom from FMD following an outbreak caused by a pig-adapted strain of FMD virus (FMDV) should differ significantly from an application designed to prove freedom from FMD for a country or zone where African buffaloes (*Syncerus caffer*) provide a potential reservoir of infection. It is incumbent upon the applicant country to submit a dossier to the OIE in support of its application that not only explains the epidemiology of FMD in the region concerned but also demonstrates how all the risk factors are

managed. This should include provision of scientifically based supporting data. There is therefore considerable latitude available to Member Countries to provide a well-reasoned argument to prove that the absence of FMDV infection (in non-vaccinated populations) or circulation (in vaccinated populations) is assured at an acceptable level of confidence.

Surveillance for FMD ~~may~~ should be in the form of a continuing ~~disease surveillance~~ programme ~~or it may be a specific programme~~ designed to establish that the whole territory or part of it is free from FMDV infection/circulation.

For the purpose of this Appendix, virus circulation means transmission of FMDV as demonstrated by clinical signs, serological evidence or virus isolation.

Article 3.8.~~6~~7.2.

General conditions and methods

1) A surveillance system in accordance with Appendix 3.8.1 should be under the responsibility of the *Veterinary Administration*. A procedure should be in place for the rapid collection and transport of samples from suspect cases of FMD to a laboratory for FMD diagnoses as described in the *Terrestrial Manual.*

2) The FMD surveillance programme should:

   a) include an early warning system throughout the production, marketing and processing chain for reporting suspicious cases. Farmers and workers who have day-to-day contact with livestock, as well as diagnosticians, should ~~be encouraged to~~ report promptly any suspicion of FMD ~~clinical disease resembling FMD~~. They should be supported directly or indirectly (e.g. through private veterinarians or veterinary para-professionals) by government information programmes and the *Veterinary Administration*. All suspect cases of FMD should be investigated immediately. Where suspicion cannot be resolved by epidemiological and clinical investigation, samples should be taken and submitted to an approved laboratory ~~and, if still considered suspect, samples should be taken and submitted to an approved laboratory~~. This requires that sampling kits and other equipment are available for those responsible for surveillance. Personnel responsible for surveillance should be able to call for assistance from a team with expertise in FMD diagnosis and control;

   b) implement, when relevant, regular and frequent clinical inspection and serological testing of high-risk groups of animals, such as those adjacent to an FMD infected country or zone (for example, bordering a game park in which infected wildlife are present).

   An effective surveillance system will periodically identify suspicious cases that require follow up and investigation to confirm or exclude that the cause of the condition is FMDV. The rate at which such suspicious cases are likely to occur will differ between epidemiological situations and cannot therefore be predicted reliably. Applications for freedom from FMDV infection/circulation should, in consequence, provide details of the occurrence of suspicious cases and how they were investigated and dealt with. This should include the results of laboratory testing and the control measures to which the animals concerned were subjected during the investigation (quarantine, movement stand-still orders, etc.).

   ~~During investigation into suspected *outbreaks* of FMD, it is necessary to apply measures that will contain the infection to its original locality until such time as the diagnosis is confirmed or refuted, e.g. through application of quarantine measures. The details of actions that need to be applied in such situations are not covered by this guide.~~

~~3) These general requirements apply in all Member Countries submitting their annual request for re-confirmation of FMD free status although active surveillance for FMD is not a requirement for countries that are recognised by the OIE as being free from FMD without vaccination. An active surveillance programme is required from Member Countries applying for the first time for recognition of freedom from FMD for the whole country or zone either with or without vaccination. It is also a requirement for countries seeking recognition for the recovery of their former status following an *outbreak*.~~

Article 3.8.~~6~~7.2.bis

Surveillance strategies

The target population for surveillance aimed at identification of *disease* and *infection* should cover all the susceptible species within the country or zone to be recognised as free from FMDV infection/circulation.

The strategy employed may be based on randomised sampling requiring surveillance consistent with demonstrating the absence of FMDV *infection*/circulation at an acceptable level of statistical confidence. The frequency of sampling should be dependent on the epidemiological situation. Targeted surveillance (e.g. based on the increased likelihood of *infection* in particular localities or species) may be an appropriate strategy. The applicant country should justify the surveillance strategy chosen as adequate to detect the presence of FMDV infection/circulation in accordance with Appendix 3.8.1. and the epidemiological situation. It may, for example, be appropriate to target clinical surveillance at particular species likely to exhibit clear clinical signs (e.g. cattle and pigs). If a Member Country wishes to apply for recognition of a specific *zone* or *compartment* within the country as being free from FMDV infection/circulation, the design of the survey and the basis for the sampling process would need to be aimed at the population within the *zone* or *compartment*.

For random surveys, the design of the sampling strategy will need to incorporate an epidemiologically appropriate design prevalence. The sample size selected for testing will need to be large enough to detect infection/circulation if it were to occur at a predetermined minimum rate. The sample size and expected disease prevalence determine the level of confidence in the results of the survey. The applicant country must justify the choice of design prevalence and confidence level based on the objectives of surveillance and the epidemiological situation, in accordance with Appendix 3.8.1. Selection of the design prevalence in particular clearly needs to be based on the prevailing or historical epidemiological situation.

Irrespective of the survey design selected, the sensitivity and specificity of the diagnostic tests employed are key factors in the design, sample size determination and interpretation of the results obtained. Ideally, the sensitivity and specificity of the tests used should be validated for the vaccination/infection history and production class of animals in the target population.

Irrespective of the testing system employed, surveillance design should anticipate the occurrence of false positive reactions. If the characteristics of the testing system are known, the rate at which these false positives are likely to occur can be calculated in advance. There needs to be an effective procedure for following up positives to ultimately determine with a high level of confidence, whether they are indicative of infection/circulation or not. This should involve both supplementary tests and follow-up investigation to collect diagnostic material from the original sampling unit as well as herds which may be epidemiologically linked to it.

The principles involved in surveillance for *disease*/*infection* are technically well defined. The design of surveillance programmes to prove the absence of FMDV infection/circulation needs to be carefully followed to avoid producing results that are either insufficiently reliable to be accepted by the OIE or

international trading partners, or excessively costly and logistically complicated. The design of any surveillance programme, therefore, requires inputs from professionals competent and experienced in this field.

1) Clinical surveillance

Clinical surveillance aims at the detection of clinical signs of FMD by close physical examination of susceptible animals. Whereas significant emphasis is placed on the diagnostic value of mass serological screening, surveillance based on clinical inspection should not be underrated. It may be able to provide a high level of confidence of detection of disease if a sufficiently large number of clinically susceptible animals is examined.

Clinical surveillance and laboratory testing should always be applied in series to clarify the status of FMD suspects detected by either of these complementary diagnostic approaches. Laboratory testing may confirm clinical suspicion, while clinical surveillance may contribute to confirmation of positive serology. Any sampling unit within which suspicious animals are detected should be classified as infected until contrary evidence is produced.

A number of issues must be considered in clinical surveillance for FMD. The often underestimated labour intensity and the logistical difficulties involved in conducting clinical examinations should not be underestimated and should be taken into account.

Identification of clinical cases is fundamental to FMD surveillance. Establishment of the molecular, antigenic and other biological characteristics of the causative virus, as well as its source, is dependent upon disclosure of such animals. It is essential that FMDV isolates are sent regularly to the regional reference laboratory for genetic and antigenic characterization.

2) Virological surveillance

Virological surveillance using tests described in the *Terrestrial Manual* should be conducted:

a) to monitor at risk populations;

b) to confirm clinically suspect cases;

c) to follow up positive serological results;

d) to test "normal" daily mortality, to ensure early detection of infection in the face of vaccination or in establishments epidemiologically linked to an outbreak.

3) Serological surveillance

Serological surveillance aims at the detection of antibodies against FMDV. Positive FMDV antibody test results can have four possible causes:

a) natural infection with FMDV;

b) vaccination against FMD;

c) maternal antibodies derived from an immune dam (maternal antibodies in cattle are usually found only up to 6 months of age but in some individuals and in some species, maternal antibodies can be detected for considerably longer periods);

d) heterophile (cross) reactions.

It is important that serological tests, where applicable, contain antigens appropriate for detecting antibodies against viral variants (types, subtypes, lineages, topotypes, etc.) that have recently occurred in the region concerned. Where the probable identity of FMDVs is unknown or where exotic viruses are suspected to be present, tests able to detect representatives of all serotypes should be employed (e.g. tests based on nonstructural viral proteins – see below).

It may be possible to use serum collected for other survey purposes for FMD surveillance. However, the principles of survey design described in this Appendix and the requirement for a statistically valid survey for the presence of FMDV should not be compromised.

The discovery of clustering of seropositive reactions should be foreseen. It may reflect any of a series of events, including but not limited to the demographics of the population sampled, vaccinal exposure or the presence of field strain infection. As clustering may signal field strain infection, the investigation of all instances must be incorporated in the survey design. If vaccination cannot be excluded as the cause of positive serological reactions, diagnostic methods should be employed that detect the presence of antibodies to nonstructural proteins (NSPs) of FMDVs as described in the *Terrestrial Manual.*

The results of random or targeted serological surveys are important in providing reliable evidence that FMDV infection is not present in a country or zone. It is therefore essential that the survey be thoroughly documented.

Article 3.8.~~6~~7.3.

Documentation of FMD free status

Countries applying for freedom from FMD for the whole country or a zone/compartment where vaccination is not practised

~~1)~~ ~~Introduction~~

~~A Member Country applying for recognition of freedom for the country or a zone from FMD where vaccination is not practised should provide evidence for the existence of an effective surveillance programme. The strategy and design of the surveillance programme will depend on the prevailing epidemiological circumstances. Conventionally, a statistically significant proportion of the whole population should be subjected to clinical and serological surveillance to demonstrate absence of FMDV, i.e. circulation of virus, during the preceding 12 months. This requires the support of a national or other laboratory able to undertake identification of FMDV infection through virus/antigen/genome detection and antibody tests described in the *Terrestrial Manual.*~~

~~2)~~ ~~Survey design~~

~~The target population for surveillance aimed at identification of *disease* and *infection* should cover all the susceptible species within the country or zone to be recognised as free from infection. This would usually require stratification of different species.~~

~~Countries wishing to show freedom from FMDV infection in which a pig-adapted strain of virus had been prevalent should concentrate on sampling the national pig population. However, it would also be necessary to show that no spill-over into other susceptible species has occurred. In countries or zones in which an African buffalo population is present, the buffaloes should~~

also be sampled if included in the proposed FMDV infection-free zone.

The strategy employed may be based either on randomised sampling requiring surveillance consistent with demonstrating the absence of *infection* at an acceptable level of statistical confidence. The frequency of sampling would be dependent on the epidemiological situation, but should occur at least once during the year preceding the application. Alternatively, targeted surveillance (e.g. based on the likelihood of infection in particular localities or species) may provide a more appropriate and cost effective strategy. If the latter approach is used, it would be incumbent upon the applicant country to show that the surveillance conducted was at least as effective as randomised surveillance with stratification of different susceptible species. It may, for example, be appropriate to target clinical surveillance at particular species likely to exhibit clear clinical signs (e.g. cattle and pigs) while directing serological surveillance at species that tend to develop less obvious signs of infection such as sheep and, in some locations, goats and wildlife species.

If a Member Country wishes to apply for recognition of a specific zone/region within the country as being free from FMDV infection, the design of the survey and the basis for the sampling process would need to be aimed at the population within the zone/region.

For randomised surveillance, the design of the sampling strategy will need to incorporate an epidemiologically appropriate design prevalence because, obviously, the sample selected for testing will need to be large enough to detect infection if it were to occur at a predetermined minimum rate. The sample size and expected disease prevalence determine the level of confidence in the result of the survey. A typical random sampling strategy would be one that provides 95% probability of detecting evidence of FMD or FMDV infection if it were present in 1% of the primary sampling units. A minimum expected level of infection within sampling units also has to be set to ensure that a sufficient number of animals within each sampling unit is tested to detect the infection if it were present in the sampling unit. Typically this value is set somewhere between 5-20% with a confidence level of 95%. In many instances it could be safely assumed that within-sampling unit prevalence would be greater than 5% bearing in mind the contagiousness of FMDV. Selection of the prevalence estimate clearly needs to be based on the prevailing or historical epidemiological situation. The reasoning used in the selection of prevalence parameters needs to be clearly spelt out in the dossier supplied to the OIE when applications are made for recognition of freedom from FMD.

The sensitivities and specificities of the testing methods employed also affect the design of sampling strategies. Clinical inspection, for example, typically has low sensitivity, especially in species that tend to suffer mild or indistinct signs of FMD (e.g. sheep). In other words, the probability of detecting FMD infection through identification of clinical cases is not particularly dependable and this therefore needs to be allowed for in the sampling design. For proving absence of infection through serology, it is usually desirable to have either a test with both high sensitivity (likely to detect a high proportion of seropositive individuals) and specificity (few false positive animals likely to be identified) or to use a combination of tests that together provide high net sensitivity and specificity. However, even if the net specificity is high, in cases where the design prevalence is low (e.g. in situations where proving absence of FMD is the objective), the positive predictive value (PV) of a test or testing system may be considerably lower than 100% (because PV is mainly a function of specificity and prevalence). This means that in such circumstances it needs to be anticipated that false positive results will occur. If the characteristics of the testing system are known, the rate at which these false positive are likely to occur can be calculated. In such circumstances detected prevalence rates significantly greater than the calculated rate would be suspicious of infection. More typically, the parameters of the testing system are imprecisely known and therefore an element of judgement in the interpretation of serological results will be necessary. Whatever the case, there needs to be an

effective procedure for following up serological positives to determine ultimately, to a high level of probability, whether they are indicative of infection or not. This should involve both supplementary laboratory tests (see below) and further field follow up to collect diagnostic material from the original sampling unit if possible as well as animals in the vicinity which may be epidemiologically linked to the suspect focus.

It is evident from the above that although the principles involved in surveillance for disease/infection are reasonably straight forward, design of large surveillance programmes to prove absence of FMD needs to be carefully done to avoid producing results that are either insufficiently reliable to be accepted by the OIE or international trading partners or excessively costly and logistically complicated. The design of any large surveillance programme therefore requires inputs from competent and experienced professionals in this field.

3) Clinical surveillance

Clinical surveillance aims at the detection of clinical signs of FMD by close inspection of susceptible animals. It is essential that all animals within the selected primary sampling unit are examined for signs of FMD. Any unit where suspicious animals are detected should be classified as infected until contrary evidence is produced.

There are a number of issues that need to be considered in clinical surveillance for FMD. Some of these (e.g. the general insensitivity of clinical surveillance and species differences) have been mentioned above. The practical difficulty, hard work and boredom involved in conducting repetitive clinical examinations are almost invariably underestimated (hence the low sensitivity). This therefore needs to be borne in mind in the surveillance design.

Furthermore, now that the emphasis of the chapter of this *Terrestrial Code* on FMD is on detection of infection rather than disease, it needs to be remembered that in practice detection of disease is only one of the ways in which infection can be identified. Other techniques, such as serology, may be more sensitive especially in situations where vaccination is not practised but, on the other hand, identification of clinical cases is still fundamental to FMD surveillance. Identification of such cases is also vital in providing sources of the causative virus that enable the molecular, antigenic and other biological characteristics of the virus to be established. It is essential that FMDV isolates are sent regularly to the regional reference laboratory for genetic and antigenic characterization.

4) Serological surveillance

Serological surveillance aims at the detection of antibodies against FMDV. Positive tests for FMDV antibody tests can have four possible causes:

a) natural infection with FMDV;

b) vaccination against FMD;

c) maternal antibodies derived from an immune dam (maternal antibodies in cattle are usually found only up to 6 months of age, however, in some individuals and in buffalo calves, maternal antibody can be detected for considerably longer);

d) heterophile (cross) reactions.

It is important that serological tests, where appropriate, contain antigens appropriate for detecting viral variants (types, subtypes, lineages, topotypes, etc.) that have recently occurred in

~~the region concerned. Where the probable identity of FMDVs is unknown or where exotic viruses are suspected to be present, tests able to detect representatives of all serotypes should be employed (e.g. tests based on nonstructural viral proteins – see below).~~

~~It may be possible to use serum collected for other survey purposes for FMD surveillance but the requirement for a statistically valid survey for the presence of FMDV should not be compromised.~~

~~General considerations in the design and conduct of sero surveys have been addressed above (see Survey design). An important issue requiring planning is the procedure to be followed in the event that seropositives are detected. As already indicated, it is likely that where the design prevalence is low false positive results should be anticipated. When these occur, both laboratory and field follow-up are necessary to differentiate between true and false positives.~~

~~Infected animals are unlikely to be evenly dispersed within the population and a cross-sectional analysis will usually detect clusters of infection. FMD is no exception to this general rule. Therefore, it is important to identify clusters of seropositive animals through simple mapping or more sophisticated cluster analysis.~~

~~If vaccination cannot be excluded as the cause of positive serological reactions, testing for the presence of antibodies to the nonstructural proteins (NSPs) of FMDVs (as described in the *Terrestrial Manual*) should be used.~~

~~The results of random sample or targeted surveys based on serology are important in providing reliable evidence that no FMDV infection is present in a country or zone. It is therefore essential that the survey be thoroughly documented.~~

In addition to the general conditions described in Chapter 2.2.10., a Member Country applying for recognition of FMD freedom for the country or a *zone/compartment* where vaccination is not practised should provide evidence for the existence of an effective surveillance programme. The strategy and design of the surveillance programme will depend on the prevailing epidemiological circumstances and will be planned and implemented according to general conditions and methods in this Appendix, to demonstrate absence of FMDV infection, during the preceding 12 months in susceptible populations. This requires the support of a national or other laboratory able to undertake identification of FMDV infection through virus/antigen/genome detection and antibody tests described in the *Terrestrial Manual.*

Article 3.8.~~6~~7.4.

Countries, zones or compartments applying for freedom from FMD where vaccination is practised

~~In addition to the general conditions, a country or zone applying for recognition of freedom from FMD with vaccination should show evidence of an effective surveillance programme for clinical disease and demonstrate that FMD has not occurred in the country or zone for the past 2 years. Furthermore, surveillance for FMDV infection should show that FMDV has not been circulating in the vaccinated population within the past 12 months. This will require serological surveillance incorporating tests able to detect antibodies to NSPs as described in Article 3.8.6.6.~~

In addition to the general conditions described in Chapter 2.2.10., a Member Country applying for recognition of country or *zone/compartment* freedom from FMD with vaccination should show evidence of an effective surveillance programme planned and implemented according to general conditions and methods in this Appendix. Absence of clinical disease in the country, *zone* or

compartment for the past 2 years should be demonstrated. Furthermore, surveillance should demonstrate that FMDV has not been circulating in any susceptible population during the past 12 months. This will require serological surveillance incorporating tests able to detect antibodies to NSPs as described in the *Terrestrial Manual*. Vaccination to prevent the transmission of FMDV may be part of a disease control programme. The level of herd immunity required to prevent transmission will depend on the size, composition (e.g. species) and density of the susceptible population. It is therefore impossible to be prescriptive. However, in general the aim should be to vaccinate at least 80% of the susceptible population. The vaccine must comply with the *Terrestrial Manual*. Based on the epidemiology of FMD in the country, *zone* or *compartment*, it may be that a decision is reached to vaccinate only certain species or other subsets of the total susceptible population. In that case, the rationale should be contained within the dossier accompanying the application to the OIE for recognition of status.

Evidence to show the effectiveness of the vaccination programme ~~is recommended~~ should be provided.

Article 3.8.~~6~~7.5.

Countries, *zones* or *compartments* re-applying for freedom from FMD where vaccination is either practised or not practised, following an outbreak

In addition to the general conditions described in Chapter 2.2.10., a country re-applying for country, *zone* or *compartment* freedom from FMD where vaccination is practised or not practised should show evidence of an active surveillance programme for FMD as well as absence of FMDV infection/circulation. This will require serological surveillance incorporating, in the case of a country, *zone* or *compartment* practising vaccination, tests able to detect antibodies to NSPs as described in the *Terrestrial Manual*. ~~This is particularly important if a country intends for the whole of its territory or a zone to avail itself of the possibility of a reduced waiting period, i.e. less than 2 years after the last *outbreak*.~~

Four strategies are recognised by the OIE in a programme to eradicate FMDV infection following an *outbreak*:

1) slaughter of all clinically affected and in-contact susceptible animals;

2) slaughter of all clinically affected and in-contact susceptible animals and vaccination of at-risk animals, with subsequent slaughter of vaccinated animals;

3) slaughter of all clinically affected and in-contact susceptible animals and vaccination of at-risk animals, without subsequent slaughter of vaccinated animals;

4) vaccination used without slaughter of affected animals or subsequent slaughter of vaccinated animals.

The time periods before which an application can be made for re-instatement of freedom from FMD depends on which of these alternatives is followed. The time periods are prescribed in Article 2.~~1~~2.10.7.

In all circumstances, a Member Country re-applying for country, *zone* or *compartment* freedom from FMD with vaccination or without vaccination ~~in a country or zone~~ should report the results of an active surveillance programme implemented according to general conditions and methods in this Appendix ~~in which the FMD susceptible population undergoes regular clinical examination or where active surveillance has targeted a statistically significant sample of the susceptible population. In addition, a statistically significant sample, based on the susceptible population at risk during the *outbreak*, would need to be tested for absence of FMDV infection. In particular circumstances,~~

~~targeted surveillance could be used to accomplish the task. The procedures are outlined above.~~

Article 3.8.~~6~~7.6.

The use and interpretation of serological tests (see Fig 1)

The recommended serological tests for FMD surveillance are described in the *Terrestrial Manual.*

~~ELISAs based on structural proteins are useful for screening sera for evidence of infection in animals that have not been vaccinated. However, although their sensitivity is generally high, their specificity, particularly in the case of the liquid phase blocking ELISA (LPBE), is relatively low. This presents difficulties when it comes to proving freedom from infection. These tests are also effective for monitoring serological responses to vaccination where it is certain that the animals concerned have not been infected. The net specificity of serological screening with ELISAs can be improved by retesting positive sera using the virus neutralisation test (VNT). Precise values for sensitivity and specificity of these tests are not available and, in any case, are likely to vary slightly between laboratories.~~

~~Any animal whose serum is positive by the VNT should be tested additionally for evidence of infection using either serological tests for antibodies to NSPs and/or by collection of oesophageal-pharyngeal material (probang testing) for virus detection on cell cultures or by PCR. Ideally, fresh serum should be collected from the animal(s) concerned because repeated freezing and thawing of stored sera tends to damage immunoglobulins.~~

~~Animals that have been vaccinated will have antibodies to the structural proteins of FMD virus, and some may have antibodies to the NSPs, depending on the number of times they have been vaccinated, and the amount of the NSPs present in the vaccine used. However, animals that have recovered from infection with FMD virus will have high levels of antibody to the NSPs. There are eight NSPs associated with the replication of FMD virus, namely L, 2A, 2B, 2C, 3A, 3B, 3C and 3D, and antibodies can be found to all of these in most recovered animals. Some do not persist for more than a few months, and some animals may fail to produce detectable levels to all NSPs. ELISAs have been developed to detect 2C, 3B or 3ABC antibodies, the former being detectable for up to one year after infection, and the latter for up to 2 years. A western blot technique (EITB) may also be used to detect the NSP antibodies to 2C, 3ABC, 3A, 3B and 3D; it is particularly specific and sensitive in identifying previously infected animals. All these tests have been extensively used in cattle. Similar testing in other species is on-going.~~

~~There is the option to use the NSP antibody test together with tests for detection of antibody to structural viral proteins, particularly in areas where vaccination has been used and virus activity is suspected. Titres higher than would be expected from vaccination alone may suggest FMDV infection and this can be confirmed by testing for the presence of antibodies to the NSPs.~~

~~As indicated above, the diagnostic sensitivity of tests used influences the numbers of animals that need to be sampled in a survey to provide evidence of absence of infection. The diagnostic specificity of the test influences the proportion and number of positive results to be expected in the absence or presence of infection, and therefore the selection and use of confirmatory tests. Results of surveys which indicate a significantly higher proportion of positive test results in comparison with that expected from the estimate of the false positive rate derived from the diagnostic specificity (i.e. 100 minus diagnostic specificity) may be interpreted as evidence of infection in the population. A confirmatory test of high specificity, and where appropriate other investigations, should be conducted to prove or refute the possibility of infection.~~

~~Figure 1 provides a flowchart of the test protocol that could be used to test the samples collected in a serological survey. If the population being tested has not been previously vaccinated against FMD,~~

~~the serum samples can be tested using ELISAs based on structural proteins. Sera positive on the test used should be retested using the VNT, which increases the net specificity. In addition, or in place of the VNT if the laboratory is not able to manipulate live FMDV, the positive sera may be retested using an NSP antibody test, such as the 3B, 3ABC or EITB. A positive VNT or NSP test would suggest that live virus had been circulating, and would require further investigation of the herd or flock to confirm or refute the possibility. Further investigation should include serum testing of the whole herd or flock from which the positive samples were obtained. NSP tests should be used for testing sera from vaccinated herds or flocks, as such sera will be positive by VNT. 3ABC or 3B positive samples may be repeat tested using the EITB for confirmation. All animals from the unit from which positive samples are obtained should be re-tested for antibodies to NSPs.~~

~~The sensitivity and specificity of the NSP tests currently available are not fully documented, in particular for species other than cattle. Member Countries submitting to the OIE data derived from commercial or other NSP tests should provide information on the characteristics of the test being used.~~

Animals infected with FMDV produce antibodies to both the structural proteins (SP) and the nonstructural proteins (NSP) of the virus. Tests for SP antibodies to include SP-ELISAs and the virus neutralisation test (VNT). The SP tests are serotype specific and for optimal sensitivity should utilise an antigen or virus closely related to the field strain against which antibodies are being sought. Tests for NSP antibodies include NSP I-ELISA 3ABC and the electro-immunotransfer blotting technique (EITB) as recommended in the *Terrestrial Manual* or equivalent validated tests. In contrast to SP tests, NSP tests can detect antibodies to all serotypes of FMD virus. Animals vaccinated and subsequently infected with FMD virus develop antibodies to NSPs, but in some, the titre may be lower than that found in infected animals that have not been vaccinated. Both the NSP I-ELISA 3ABC and EITB tests have been extensively used in cattle. Validation in other species is ongoing. Vaccines used should comply with the standards of the *Terrestrial Manual* insofar as purity is concerned to avoid interference with NSP antibody testing.

Serological testing is a suitable tool for FMD surveillance. The choice of a serosurveillance system will depend on, amongst other things, the vaccination status of the country. A country, which is free from FMD without vaccination, may choose serosurveillance of high-risk subpopulations (e.g. based on geographical risk for exposure to FMDV). SP tests may be used in such situations for screening sera for evidence of FMDV infection/circulation if a particular virus of serious threat has been identified and is well characterised. In other cases, NSP testing is recommended in order to cover a broader range of strains and even serotypes. In both cases, serological testing can provide additional support to clinical surveillance. Regardless of whether SP or NSP tests are used in countries that do not vaccinate, a diagnostic follow-up protocol should be in place to resolve any presumptive positive serological test results.

In areas where animals have been vaccinated, SP antibody tests may be used to monitor the serological response to the vaccination. However, NSP antibody tests should be used to monitor for FMDV infection/circulation. NSP-ELISAs may be used for screening sera for evidence of infection/circulation irrespective of the vaccination status of the animal. All herds with seropositive reactors should be investigated. Epidemiological and supplementary laboratory investigation results should document the status of FMDV infection/circulation for each positive herd. Tests used for confirmation should be of high diagnostic specificity to eliminate as many false positive screening test reactors as possible. The diagnostic sensitivity of the confirmatory test should aproach that of the screening test. The EITB or another OIE-accepted test should be used for confirmation.

Information should be provided on the protocols, reagents, performance characteristics and validation of all tests used.

1) **The follow up procedure in case of positive test results if no vaccination is used in order to establish or re-establish FMD free status without vaccination**

Any positive test result (regardless of whether SP or NSP tests were used) should be followed up immediately using appropriate clinical, epidemiological, serological and where possible virological investigations of the reactor animal at hand, of susceptible animals of the same epidemiological unit and of susceptible animals that have been in contact or otherwise epidemiologically associated with the reactor animal. If the follow up investigations provide no evidence for FMDV infection, the reactor animal shall be classified as FMD negative. In all other cases, including the absence of such follow up investigations, the reactor animal should be classified as FMD positive.

2) **The follow up procedure in case of positive test results if vaccination is used in order to establish or re-establish FMD free status with vaccination**

In case of vaccinated populations one has to exclude that positive test results are indicative of virus circulation. To this end the following procedure should be followed in the investigation of positive serological test results derived from surveillance conducted on FMD vaccinated populations.

The investigation should examine all evidence that might confirm or refute the hypothesis that the positive results to the serological tests employed in the initial survey were not due to virus circulation. All the epidemiological information should be substantiated and the results should be collated in the final report.

It is suggested that in the primary sampling units where at least one animal reacts positive to the NSP test, the following strategy(ies) should be applied:

a) Following clinical examination, a second serum sample should be taken from the animals tested in the initial survey after an adequate interval of time has lapsed, on the condition that they are individually identified, accessible and have not been vaccinated during this period. Antibody titres against NSP at the time of retest should be statistically either equal to or lower than those observed in the initial test if virus is not circulating.

   The animals sampled should remain in the holding pending test results and should be clearly identifiable. If the three conditions for retesting mentioned above cannot be met, a new serological survey should be carried out in the holding after an adequate period of time, repeating the application of the primary survey design and ensuring that all animals tested are individually identified. These animals should remain in the holding and should not be vaccinated, so that they can be retested after an adequate period of time.

b) Following clinical examination, serum samples should be collected from representative numbers of cattle that were in physical contact with the primary sampling unit. The magnitude and prevalence of antibody reactivity observed should not differ in a statistically significant manner from that of the primary sample if virus is not circulating.

c) Following clinical examination, epidemiologically linked herds should be serologically tested and satisfactory results should be achieved if virus is not circulating.

d) Sentinel animals can also be used. These can be young, unvaccinated animals or animals in which maternally conferred immunity has lapsed and belonging to the same species resident within the positive initial sampling units. They should be serologically negative if virus is not circulating. If other susceptible, unvaccinated ruminants (sheep, goats) are present, they could act as sentinels to provide additional serological evidence.

Laboratory results should be examined in the context of the epidemiological situation. Corollary information needed to complement the serological survey and assess the possibility of viral circulation includes but is not limited to:

– characterization of the existing production systems;

– results of clinical surveillance of the suspects and their cohorts;

– quantification of vaccinations performed on the affected sites;

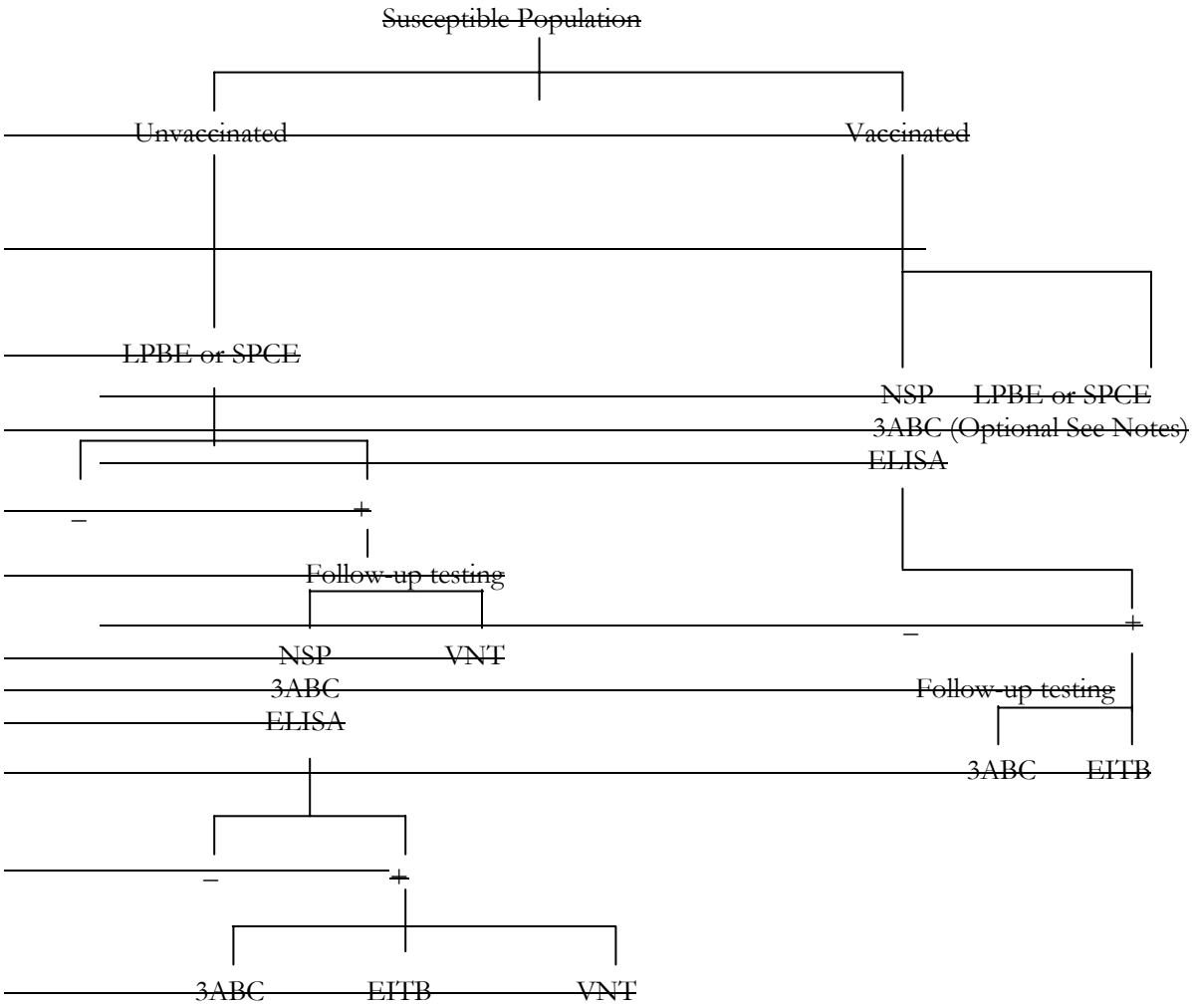
– sanitary protocol and history of the establishments with positive reactors;

– control of animal identification and movements;

– other parameters of regional significance in historic FMDV transmission.

The entire investigative process should be documented as standard operating procedure within the surveillance programme.

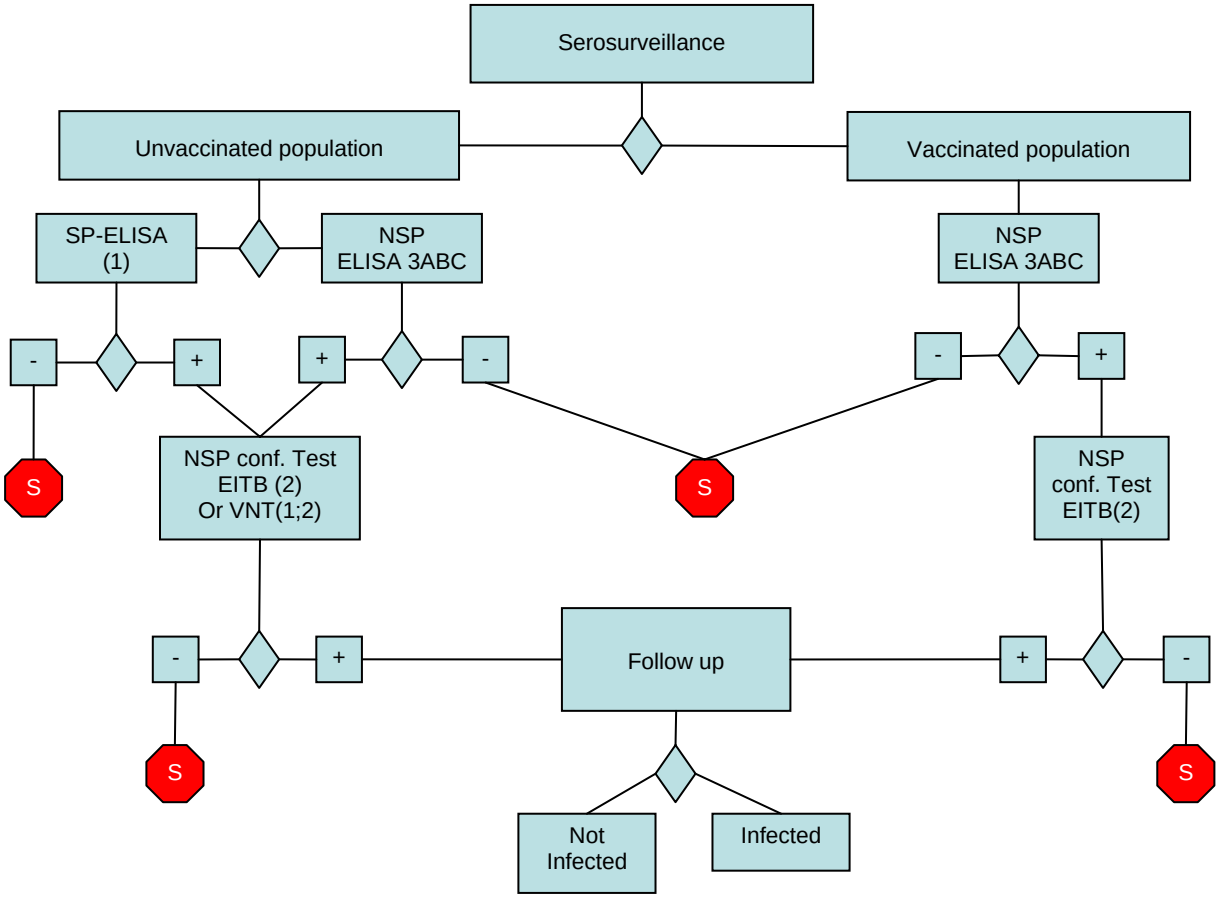
Figure 1 ***Schematic representation of laboratory tests for determining evidence of FMDV infection through or following serological surveys***



The above diagram indicates the tests which are recommended for use in the investigation of sampling units in which a positive test result has been obtained.

When feasible, detection of virus in OP fluid can also be used as complementary test on units in which positive NSP test result has been obtained.

Figure 1 ***Schematic representation of laboratory tests for determining evidence of FMDV infection through or following serological surveys***



Key:

ELISA	Enzyme-linked immunosorbent assay
VNT	Virus neutralisation test
NSP	Nonstructural protein(s) of foot and mouth disease virus (FMDV)
3ABC	NSP antibody test
EITB	Electro-immuno transfer blotting technique (Western blot for NSP antibodies of FMDV)
OP	Oesophageal–pharyngeal sample
SP	Structural protein test
S	No evidence of FMDV